#8 YC 10-1001

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

PODBIELSKI

Serial No.: 09/494,297 Examiner: N. Minnifield

Filed: January 31, 2000 Art Unit: 1645

For: COLLAGEN-BINDING PROTEINS FROM STREPTOCOCCUS PYOGENES Docket No.: P06628US0/BAS

DECLARATION UNDER 37 C.F.R. § 1.131

Assistant Commissioner for Patents
Washington, DC 20231

S I R:

I, Andreas Podbielski, declare and state as follows:

1. I am the sole inventor of the above-identified patent application. I am also the principle author of the journal article, *Characterization of nra, a global negative regulator gene in group A streptococci*, published in Molecular Microbiology (1999) 31(4), pages 1051-1064 (hereinafter the "Article").

2. The claimed subject matter which is disclosed in the Article is exclusively my own and therefore not the product of another.

3. Co-authors of the Article, Markus Woischnik, Bettina A.B. Leonard and Karl-Hermann Schmidt worked at my direction and assisted me in preparing and writing the Article but did not contribute to the subject matter of the claimed invention.

4. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements

and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Sept. 18, 2001

Prof. Dr. Dr. A. Podbielski
Arzt für Med. Mikrobiologie
und Infektionsepidemiologie

Andreas Podbielski

1: Mol Microbiol 1999 Feb;31(4):1051-1064 Related Articles, Protein, Books, Lin

Characterization of nra, a global negative regulator gene in group streptococci.

Podbielski A, Woischnik M, Leonard BA, Schmidt KH.

Department of Medical Microbiology and Hygiene, University Hospital Ulm, Germany. andreas.podbielski@medizin.uni-ulm.de

During sequencing of an 11.5 kb genomic region of a serotype M49 group A streptococcal (GAS) strain, a series of genes were identified including nra(negativ regulator of GAS). Transcriptional analysis of the region revealed that nra was primarily monocistronically transcribed. Polycistronic expression was found for th three open reading frames (ORFs) downstream and for the four ORFs upstream of The deduced Nra protein sequence exhibited 62% homology to the GAS RofA positive regulator. In contrast to RofA, Nra was found to be a negative regulator o own expression and that of the two adjacent operons by analysis of insertional inactivation mutants. By polymerase chain reaction and hybridization assays of 10 different GAS serotypes, the genomic presence of nra, rofA or both was demonstr Nra-regulated genes include the fibronectin-binding protein F2 gene (prtF2) and a novel collagen-binding protein (cpa). The Cpa polypeptide was purified as a recombinant maltose-binding protein fusion and shown to bind type I collagen but fibronectin. In accordance with nra acting as a negative regulator of prtF2 and cpa, levels of attachment of the nra mutant strain to immobilized collagen and fibronec was increased above wild-type levels. In addition, nra was also found to regulate negatively (four- to 16-fold) the global positive regulator gene, mga. Using a strai carrying a chromosomally integrated duplication of the nra 3' end and an nra-luciferase reporter gene transcriptional fusion, nra expression was observed to reach its maximum during late logarithmic growth phase, while no significant influence of atmospheric conditions could be distinguished clearly.

PMID: 10096074 [PubMed - indexed for MEDLINE]

1: J Clin Invest 1994 Sep;94(3):965-977 Related Articles, Books, Lin

M protein and protein F act as important determinants of cell-specific tropism of Streptococcus pyogenes in skin tissue.

Okada N, Pentland AP, Falk P, Caparon MG.

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110-1093.

The pathogenic gram-positive bacterium Streptococcus pyogenes (group A streptococcus) causes numerous diseases of cutaneous tissue, each of which is initiated after the interaction of the bacterium with the cells of the epidermis. I study, we show that different surface proteins of S. pyogenes play important ro determining the cell-specific tropism of the bacterium in skin. Using streptoco strains with defined mutations in the genes which encode surface proteins in combination with primary cultures of human skin and an in situ adherence assa which uses histological sections of human skin, we show that the M protein of pyogenes mediates the binding of the bacterium to keratinocytes, while a secon streptococcal surface protein, protein F, directs the adherence of the organism t Langerhans' cells. Characterization of binding revealed that adherence was inhi by purified streptococcal proteins and pretreatment of both host cells with the protease trypsin. Adherence was only slightly affected by the state of keratinoc differentiation in vitro, but was considerably modulated in response to environmental conditions known to regulate expression of M protein and prote suggesting that the interaction between these bacterial cell-surface structures/adhesins and keratinocytes and Langerhans' cells may play an impor role in streptococcal skin disease.

PMID: 8083381 [PubMed - indexed for MEDLINE]

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